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Published in:
British Journal of Anaesthesia

DOI:
10.1093/bja/69.4.382

British Journal of Anaesthesia 1992; **69**: 382–386

PORCINE MODEL FOR STUDYING THE PASSAGE OF NON-DEPOLARIZING NEUROMUSCULAR BLOCKERS THROUGH THE BLOOD–BRAIN BARRIER

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SUMMARY

A method has been developed for blood–brain barrier disruption to provide reproducible access to the cerebrospinal fluid of the cerebellomedullary cistern. The technique was used successfully to investigate transfer of pancuronium to the cerebral CSF compartment in pigs. After osmotic disruption of the blood–brain barrier, pancuronium concentrations increased significantly in the cerebrospinal fluid.

KEY WORDS

Brain: blood–brain barrier. Cerebrospinal fluid: drug transfer Neuromuscular relaxants: pancuronium.

It is accepted generally that quaternary compounds with a molecular centre carrying a strong positive charge do not cross the blood–brain barrier (BBB) in concentrations great enough to affect the brain. In spite of this, several neuromuscular blocking drugs have been shown to produce, not only EEG evidence of arousal reactions, but also decreases in frequency and amplitude in animals with disrupted BBB, which may indicate a depressant effect on the activating system of the medulla oblongata [1]. Therefore, interest has been focused on the passage of neuromuscular blockers through the supposedly compromised BBB and their possible toxic effects on cholinergic pathways in brainstem and midbrain structures [2]. If neuromuscular blocking agents do enter the CNS in large concentrations, recovery in patients with altered states of BBB might be delayed because of anticholinergic effects in midbrain structures (e.g. thalamus) [3].

There are few data on the passage of non-depolarizing neuromuscular blockers into the cerebrospinal fluid (CSF) in man [4, 5]. Furthermore, neither qualitative nor quantitative assessment of neuromuscular blocking drug penetration into the CSF before and after BBB disruption has been investigated under well controlled conditions.

The aim of this study was to develop a reliable technique for BBB disruption which also provides convenient access to the CSF of the cerebellomedullary cistern in the pig. After the model had been developed, we investigated the passage of pancuronium through the intact and disrupted BBB.

MATERIALS AND METHODS

Experimental procedure

After approval by the committee on animal research, we studied eight previously tested MH-free pigs (German breed) weighing 20–22 kg. Anaesthesia was induced by i.v. injection of thiopentone 10 mg kg^{-1} and maintained by a continuous infusion of 10–15 mg kg^{-1} h^{-1}. Additional opioids or inhalation agents were not administered. Tracheotomy was performed and ventilation using an oxygen–air mixture (FI_{O_2} 0.4) was adjusted to maintain arterial Pa_{CO_2} within normal limits (4.6–5.3 kPa) as assessed by capnography and intermittent blood-gas analysis. Ringer solution was infused i.v. at a rate of 3–5 ml kg^{-1} as required to keep haemodynamic variables within 10% of control values. In order to avoid excessive filling of the urinary bladder, suprapubic puncture and drainage was performed. An indwelling polyethylene catheter (0.9 mm i.d.) was placed in the right femoral artery for pressure recording and blood sampling. After exposure and ligation, the left common carotid artery (CCA) was cannulated (polyethylene cannula 1 mm i.d.), with the tip of the catheter just proximal to the occipital artery, for administration of hypertonic mannitol.

At first, the effects of continuous proximal CCA ligation on BBB integrity were investigated in three animals. After 60 min of CCA ligation, 2% Evan's blue 3 ml kg^{-1} was injected slowly via a peripheral vein for determination of BBB integrity. After an equilibration period of 10 min, the animal was killed with potassium chloride and the brain was removed immediately.

For cisternal catheter positioning, the animals were placed in a right lateral position with maximal flexion of the head to provide optimal access to the

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Accepted for Publication: May 6, 1992.

occipital bone. A midline incision was made from 3 cm proximal to approximately 13 cm distal to the external occipital protuberance. The muscles of the neck were separated by blunt dissection, the occipital bone exposed and the dorsal edge of the foramen magnum identified. The area between the nuchal ridge and the right atlanto–occipital junction was exposed. An 11-mm hole was drilled to give access to the dura mater; its position was selected exactly 1 cm cranial to the base of the occiput and approximately 1 cm medial to a vertical line through the right atlanto–occipital junction in order to avoid bleeding from extradural veins or from the median occipital sinus. After incision of the dura, cannulation of the cerebellomedullary cistern was performed by moving a polyethylene catheter (i.d. 0.5 mm, length 15 cm) along the dura approximately 2 cm in a medial and caudal direction. Correct position within the cisterna magna was confirmed by slow aspiration of clear CSF. Before insertion of the catheter, its tip was rounded by heating to avoid vascular lesions or damage of pial structures. Free flow of CSF was facilitated by two side holes within 5 mm of the tip. The drill hole was sealed with Histacryl to prevent both leakage of CSF and accidental removal of the catheter.

After completion of the surgical procedure and cisternal catheter placement, 25% mannitol 3 ml kg^{-1} was injected slowly over 30 s into the left CCA to induce osmotic disruption of the BBB. The degree of BBB disruption was determined by assessing the staining of the cortex subsequent to injection of Evan's blue dye at the end of each experiment.

The staining of the brain was graded as follows: grade 0 = no staining; grade 1+ = just noticeable staining; grade 2+ = moderate blue staining; grade 3+ = dark blue staining [6, 7]. Correct catheter position in the cisterna magna was checked at autopsy.

Pancuronium assay in plasma and CSF

The concentration of pancuronium in CSF before and after mannitol-induced BBB disruption and plasma concentrations were measured by high pressure liquid chromatography [8]. Plasma and internal standard were mixed with potassium iodide–glycine buffer and dichlormethane for 30 s. After centrifugation, the bottom phase was evaporated to dryness at 45 °C under a stream of nitrogen. The dry residue was reconsisted in 250 µl of the HPLC eluent for further analysis. Liquor samples were analysed without extraction. A model 116 HPLC pump with a 210 A sample injection valve (Beckman Instr.) was used. The separation was carried out on a Nova Pak C18 column (Waters–Millipore) with a mobile phase consisting of sodium dihydrogen phosphate (NaH_2PO_4) 0.1 mol $litre^{-1}$ (pH = 3.0, orthophosphoric acid (H_3PO_4) adjusted), 9,10-dimethoxyanthracene-2-sulfonate (DAS) 0.11 mmol $litre^{-1}$ heptansulfonate (HS) 0.11 mmol $litre^{-1}$ in water–dioxane (85:15 v/v) at a flow rate of 1 ml min^{-1}.

For the purpose of the on-line post-column ion pair extraction, dichloroethane was added by a 880 PU-Jasco solvent delivery system and mixed with the HPLC eluent using a small deadspace volume T-piece. The extraction was performed in a 3.2-m × 1-mm i.d. coiled Teflon capillary connected to a sandwich-type phase separator. The phase separator consisted of a PTFE disc with a 42 × 1.5-mm groove sandwiched between two stainless-steel blocks. The split ratio of the phase separator was adjusted with a microcontrol needle valve BMVC (SGE) mounted in the outlet of the upper steel block.

The fluorimetric detector (LS-2B, Perkin Elmer) was set at 385 nm (excitation) and 452 nm (emission). After each series of analyses, the HPLC-column was flushed with water (approx. 15 ml) and methanol (approx. 75 ml). The HPLC-system was operated and the chromatograms were recorded and processed with System Gold chromatography software (Beckman Instr.).

Linearity was observed ($r = 0.9956$) over a range of 0.05–10 µg ml^{-1}. The extraction recovery was approximately 30–70% compared with that of unextracted standard samples. The limit of detection for all substances (pancuronium and its metabolites) was approximately 5 ng ml^{-1}.

Pancuronium penetration into the cerebral CSF compartment before and after disruption of the blood–brain barrier

Having established the method in preliminary studies, we applied the technique in eight additional experiments.

After completion of the operative procedure, neuromuscular monitoring was commenced using supramaximal (2–4-mA) stimulation of the common peroneal nerve with stimuli of 0.2 ms duration at a rate of 0.1 Hz and anterior tibial muscle mechanomyography. After a stabilization period of 10 min, a bolus dose of pancuronium 220 µg kg^{-1} ($2 \times ED_{90}$) was given i.v. and, after spontaneous twitch recovery to 10% of control, an infusion of pancuronium was administered continuously for 60 min; the infusion rate was adjusted to keep neuromuscular block at 85–90% of control. Blood and CSF samples were obtained 2, 10, 30, 60 and 90 min after the administration of the bolus dose of pancuronium.

After complete spontaneous recovery of neuromuscular function, each animal was given 25% mannitol 3 ml kg^{-1} to disrupt the BBB, and the animal was subjected again to the programme as described above.

At the end of the experiment, Evan's blue was injected i.v.; 10 min later the animal was killed and the brain removed immediately for assessment of the level of brain staining as evidence for BBB disruption.

Statistics

Pancuronium concentrations in plasma and CSF were assessed statistically using the Wilcoxon test for paired data. Level of significance was assumed to be 95% ($P < 0.05$). Data are presented as mean values (SEM).

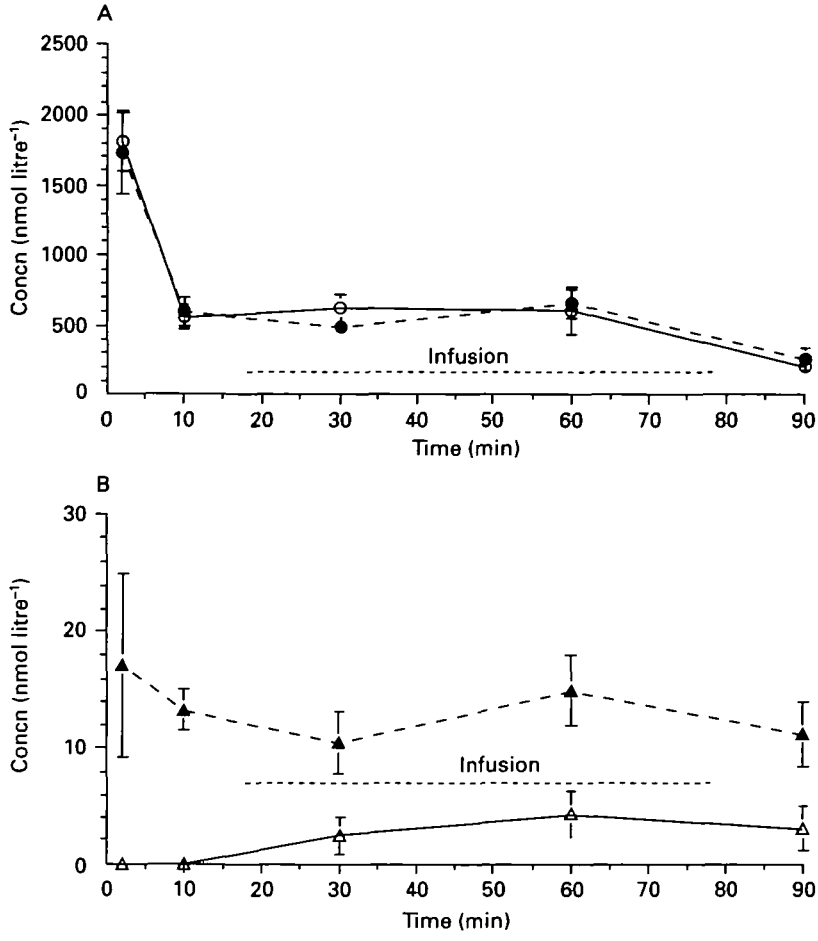

FIG. 1. Time course of mean (SEM) plasma (A) and CSF concentrations (B) of pancuronium before (○, △) and after (●, ▲) osmotic disruption of the BBB. At 0 a 2 × ED_{90} bolus dose was administered; continuous infusion from 18 to 78 min. Pancuronium concentrations in the CSF after BBB disruption were significantly greater ($P < 0.05$).

RESULTS

Haemodynamic and respiratory variables remained stable throughout all experiments.

Unilateral common carotid artery ligation

Unilateral common carotid artery ligation produced no haemodynamic effects. In the three animals subjected to this procedure, Evan's blue staining was not detected 60 min after continuous ligation of the CCA.

Access to cerebrospinal fluid

Using the transoccipital approach, clear CSF could be obtained in each case as required by the study. Correct catheter position within the centre of the cerebellomedullary cistern was confirmed at autopsy.

Osmotic blood–brain barrier disruption

In all cases, reproducible Evan's blue staining of the cortex (grade 2+ = moderate blue staining) was obtained, which suggests that the desired degree of BBB disruption was achieved with 25% mannitol 3 ml kg^{-1} injected into the CCA.

Concentration of pancuronium in the cerebrospinal fluid before and after disruption of the blood–brain barrier

There was no statistically significant difference in plasma concentrations of pancuronium before and after BBB disruption (fig. 1A). In contrast, after osmotic disruption of the BBB a significantly greater concentration of pancuronium was detected in CSF (fig. 1B). The CSF:plasma ratio was 0.023, which is significantly greater than the ratio of 0.005 before disruption of the BBB.

DISCUSSION

Osmotic disruption of the BBB by direct or retrograde infusion of hypertonic solutions into the internal carotid artery has been standardized in small rodents, monkeys and dogs [6, 9, 10]. In the pig, as a result of certain anatomical differences in arterial blood supply of the brain, only the CCA is accessible for injection of hypertonic solutions. When this approach is used for BBB disruption, continuous CCA ligation may result, not only in BBB breakdown, but also in an increased incidence of apnoea, bradycardia, hypotension or even permanent brain

damage [11]. However, our preliminary studies clearly showed that unilateral continuous CCA ligation in the pig was not followed by undesired haemodynamic responses and in no case was Evan's blue staining detected in brain, even after a 60-min period of continuous ligation. This demonstrates that, in the pig, sufficient collateral cerebral circulation is provided by the dense extradural arterial network which is supplied by both CCA [12].

In earlier studies the injection of hypertonic mannitol 1.5 ml kg^{-1} into the CCA in dogs resulted in inconsistent BBB disruption because of a large distal external to internal carotid collateral circulation via the snout [10, 13]. Taking into account the apparently larger capacity of the complex cerebro–arterial system in the pig, in the present study 25% mannitol 3 ml kg^{-1} was used to produce the desired degree of disruption. In all experiments this was followed by a moderate Evan's blue staining of both hemispheres, indicating osmotic breakdown.

Even minimal blood staining of CSF impedes reliable qualitative and quantitative assessment of drug transfer through the BBB. Although several methods of access to the cerebral ventricular system and the subarachnoid space of the cerebellomedullary cistern have already been described in the pig, frequent withdrawal of clear (not blood-stained) CSF could not be guaranteed [14, 15]. When stereotaxic insertion of the ventricular cannula is used, CSF removal is frequently unsuccessful because of ventricular collapse or blocking of the cannula by the choroid plexus. In addition, stretching and distortion of choroid vessels by suction may cause progressive bleeding and may consequently lead to unintended mechanical damage of the BBB. In the cerebellomedullary cistern, the subarachnoid space is rather small and the distance between the atlanto–occipital membrane and the medulla oblongata is usually < 5 mm in pigs weighing 20–22 kg. Thus a percutaneous technique with subsequent puncture of the atlanto–occipital membrane may not permit correct catheter positioning. A misplaced catheter may result immediately in a dry tap, disastrous puncture of the medulla oblongata or blood-stained CSF as a result of damage to pial vessels. In order to guarantee reliable access to clear CSF, a retrograde approach to the cerebellomedullary cistern has been developed and used successfully in our experiments. Meticulous placement of the occipital drill hole within the described anatomical structures and careful movement of the catheter in a medio–caudal direction has proved to be of the utmost importance.

When studying the passage of any drug through the BBB over a given period, it is essential that the amount of CSF withdrawn for analysis does not exceed its rate of production. There are no data available for the rate of CSF formation in the pig, but, assuming the rate is comparable to that in the dog, withdrawal of 0.3–0.5 ml every 10 min is reasonable [16].

The animal model described has been used successfully to study the passage of pancuronium from blood to CSF. Despite being ionized, i.v. administered pancuronium passes into the CSF in small amounts, the resulting CSF : plasma ratio being very small. However, after BBB disruption a significantly greater concentration of pancuronium was detected in CSF.

In conclusion, the pig serves as an excellent model for investigating the passage of drugs through the intact and disrupted BBB. The data obtained from this experimental model strongly suggest that i.v. administered pancuronium might yield CSF concentrations large enough to affect the CNS when the BBB is compromised. This should be considered whenever neuromuscular blocking agents or other drugs not thought to cross the BBB are administered simultaneously with high-dose mannitol, for example to enforce diuresis or to reduce rapidly an increased intracranial pressure.

ACKNOWLEDGEMENT

We are indebted to Mrs G. Köberl for HPLC analysis of plasma and CSF concentrations of pancuronium.

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